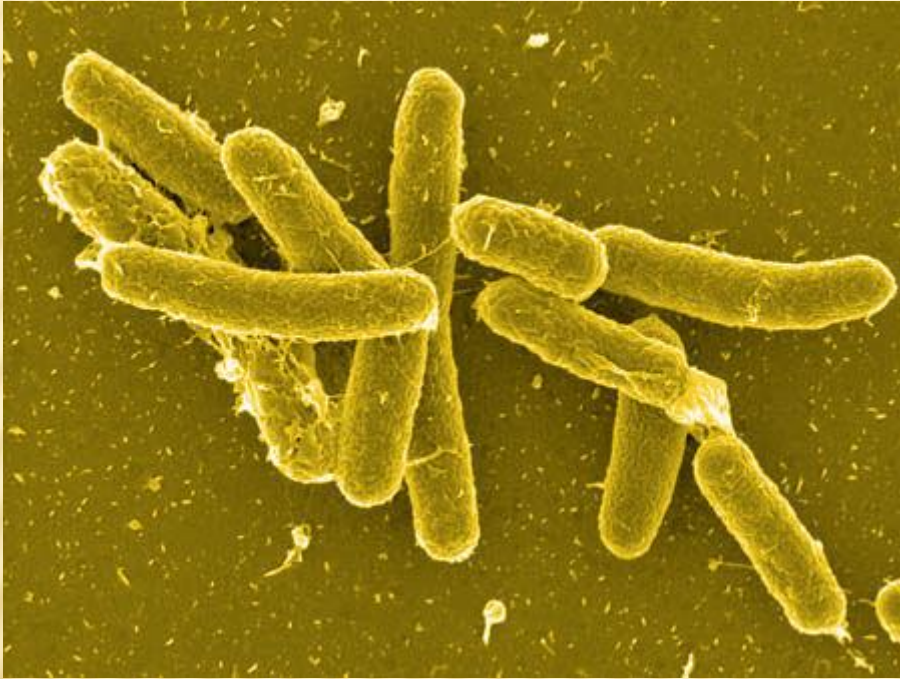**SALMONELLA**

BACKGROUND


- S. Newport PFGE pattern JJPX01.0061 historically associated with tomatoes in Delmarva Peninsula.
- CDC request all S. Newport JJPX01.0061 isolated from clinical samples from 10 states and 2 jurisdictions.
- Surveillance Period 07/01/15-10/15/15
- Internal State surveillance Program established based on recent outbreaks

DELAWARE PUBLIC HEALTH LABORATORY SURVEILLANCE PROJECT-S. NEWPORT

DPHL/CDC SURVEILLANCE PROGRAM

* CDC perform whole genome sequencing (WGS) to enhance human illness surveillance.

* Rapidly identify sub-clusters, facilitate traceback of contaminated produce, and initiate environmental assessment.

* In addition, six states (including DE) to submit **all** S. Newport from clinical sources.
  + Although distinct by PFGE, highly related by WGS
  + Part of inclusion with Epi questionnaire

FINAL 2014 EPI DATA
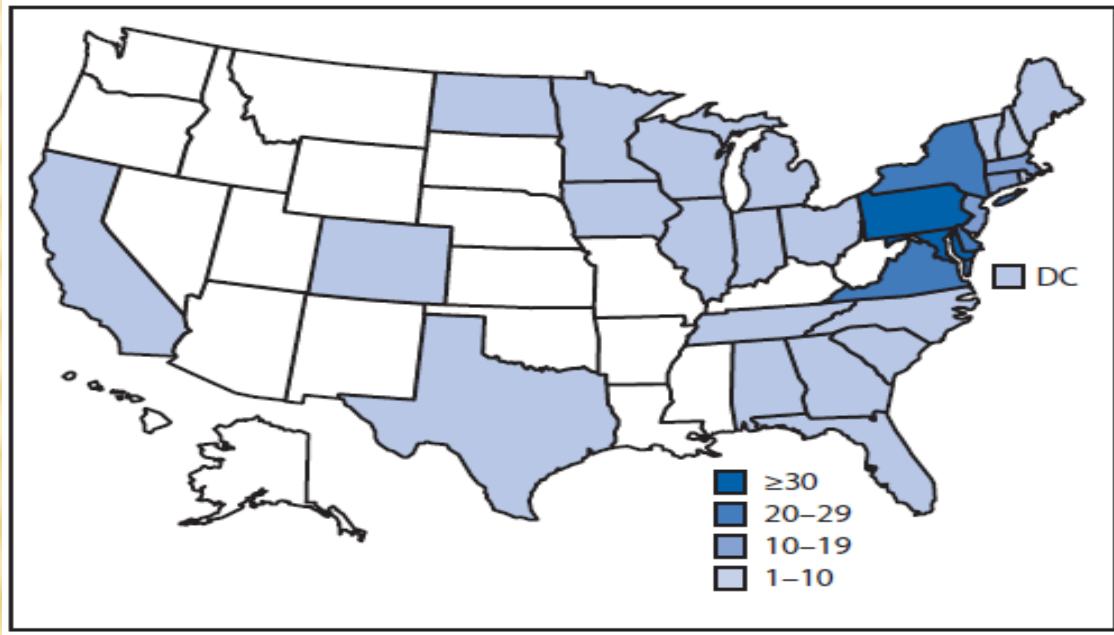
- ✖ Confirmed case definition:
  - ➕ Persons with *Salmonella* Newport infection,
  - ➕ With illness onset May 1, 2014 to Sept 29th, and
  - ➕ An isolate matching PFGE pattern JJPX01.0061
- ✖ 21 cases in DE
- ✖ 275 cases in 29 states + DC
- ✖ Estimated and reported illness onset dates
  - ➕ May 14th to Sept 30th ,2014

FINAL 2014 EPI DATA CONT.

- DPH and other States worked with our FDA District Office's foodborne outbreak rapid response teams to conduct an informational (i.e., non-regulatory) trace back for produce items (i.e., cucumbers, leafy greens, and tomatoes) consumed in nine of 12 sub-clusters.
- Establishments in MD and DE received cucumbers from a single major distributor.
- Officials collected environmental samples from the farm but no salmonella was isolated, however sampling took place months after harvesting.
- The farm noted spreading poultry litter 120 days prior to harvesting.

CDC MMWR PUBLICATION

Cases of *Salmonella* Newport in cluster 1507MLJJP-1 as of 10/2/15

Source State	Total
CT	1
DE	7
MA	2
MD	7
NC	3
NJ	12
NY	4
PA	9
RI	1
VA	6
Grand Total	**52**

- Case definition:
  - Infection with *Salmonella* Newport and
  - with isolate matching PFGE pattern *Xba*I JJPX01.0061, and
  - with illness onset (or isolation, if onset date unknown) during June 29, 2015– present
  - With residency in NC, VA, MA, DE, PA, NJ, NY, CT, RI, MD, NYC, and DC

- 52 cases from 10 states

- Isolation dates: 6/29/15- 9/12/15

- Reported onset dates: 6/22/15- 9/12/15

Exposure Information, 10/2/15

Supplemental questionnaire or other data available for (19) patients from CT(1), DE(1),NJ(7), VA(5), PA(2), RI(1),and MA(2).

Exposures

- 12/18 any tomato exposure
    - 10/17 red round (beefsteak) tomato exposure
    - 7/16 cherry tomato exposure
    - 4/14 grape tomato exposure
    - 3/14 roma tomato exposure
- 13/15 any lettuce exposure
- 10/16 cucumber exposure
- 11/16 melon exposure (cantaloupe, honeydew, watermelon)
- 9/14 blueberry exposure
- 7/13 strawberry exposure
- No common restaurants reported
- Walmart, ShopRite, Food Lion, Stop & Shop reported

DPHL/CDC PROJECT-2015

- To date, DPHL has submitted 31 S. Newport (nine with pattern JJPX01.0061)
- As of 09/30/15, 266 clinical isolates submitted, with 93 historic and contemporary food and environmental isolates.
- Study is still on-going with more isolates being received and identified daily.
- Statistical data from CDC WGS on next slides

SALMONELLA MOLECULAR BACKGROUND

- Genome is ~4.5-5.0MB
- Diverse organism, esp. S. Newport
  - Dozens of SNPs between one pattern # (e.g. .0061)
- Some strains very stable
  - Clinical matches from current study to environmental strains dating back to 2008
- Makes Epidemiological tracking very difficult
  - Ex. M. tuberculosis average 1,200 SNP difference between any two strains

Salmonella Newport – kSNP analysis
10-09-2015

- 303 clinical isolates
- 93 historic and contemporary food and environmental isolates
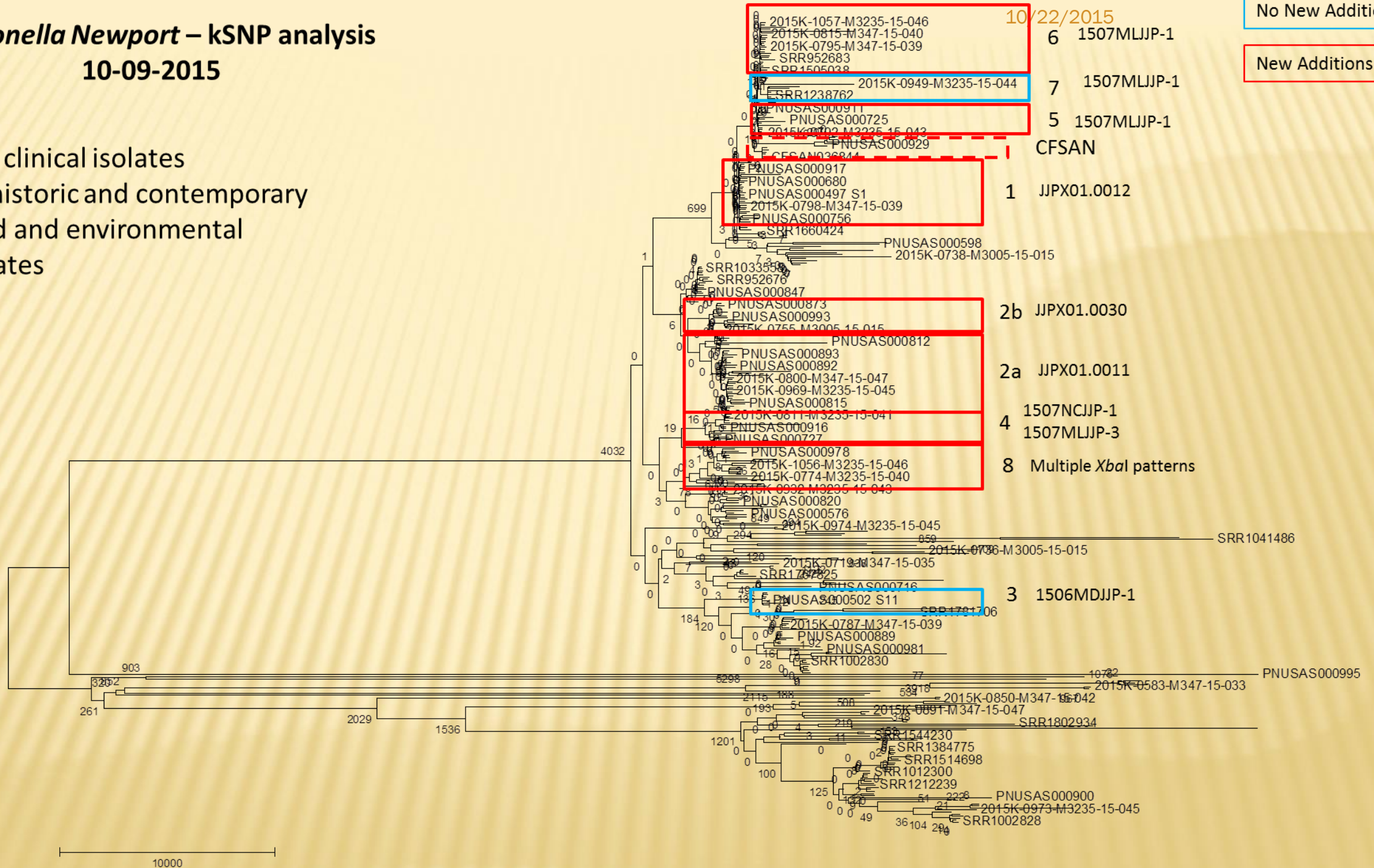
Delmarva Study - Newport hqSNP Tree- Box 1 – JJPX01.0012 cluster

Highlighted red isolates are new

Other colored isolates are different PFGE patterns

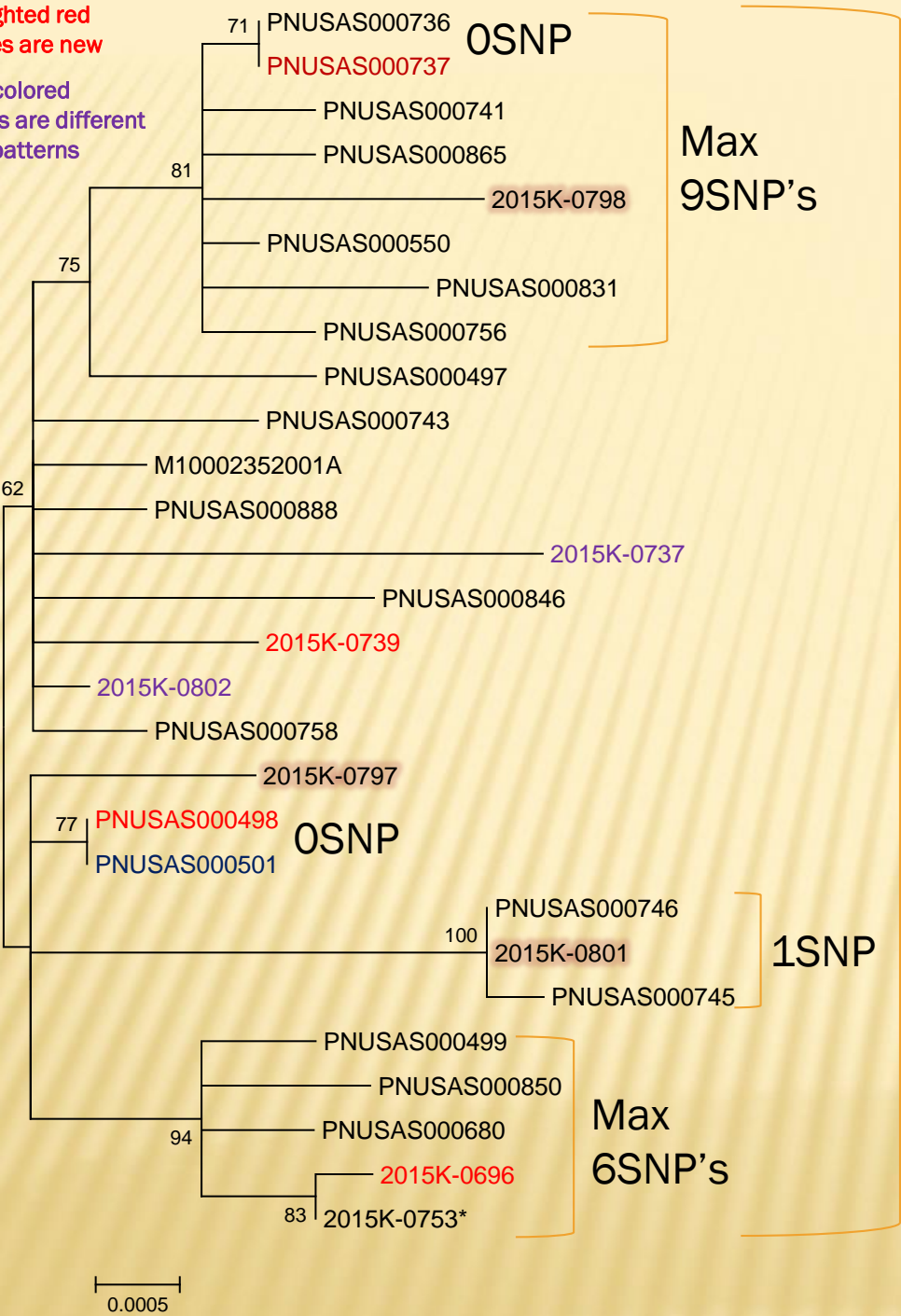| WGS_id	Key	PFGE-XbaI-pattern	Source	IsolatDate
2015K-0696	NJ___150983	JJPX01.0012	Human	7/2/2015
2015K-0737	NJ___151025	JJPX01.0061	Human	6/29/2015
2015K-0739	NJ___150903	JJPX01.0012	Human	6/20/2015
2015K-0797	DE___B629559	JJPX01.0012	Human	7/12/2015
2015K-0798	DE___B629564	JJPX01.0012	Human	7/16/2015
2015K-0801	DE___B624391	JJPX01.0012	Human	7/27/2015
2015K-0802	NC___MB072815-0051	JJPX01.0061	Human	7/24/2015
M10002352001A	PA___M10002352001A	JJPX01.0012	Chicken Breast	8/4/2009
PNUSAS000497	MD___MDA15042335	JJPX01.0012	Human	4/2/2015
PNUSAS000498	MD___MDA15063220	JJPX01.0012	Human	5/20/2015
PNUSAS000499	MD___MDA15065370	JJPX01.0012	Human	5/16/2015
PNUSAS000501	MD___MDA15063221	JJPX01.3388	Human	5/21/2015
PNUSAS000550	NY___IDR1500051271	JJPX01.0012	Human	6/27/2015
PNUSAS000680	MD___MDA15080024	JJPX01.0012	Human	6/25/2015
PNUSAS000736	NY___IDR1500055214	JJPX01.0012	Human	7/25/2015
PNUSAS000737	NY___IDR1500055402	JJPX01.0441	Human	7/30/2015
PNUSAS000741	NY___IDR1500056592	JJPX01.0012	Human	7/24/2015
PNUSAS000743	VA___R150800453	JJPX01.0012	Human	8/2/2015
PNUSAS000745	MD___MDA15088429	JJPX01.0012	Human	7/16/2015
PNUSAS000746	MD___MDA15088969	JJPX01.0012	Human	7/19/2015
PNUSAS000756	NY___IDR1500056792	JJPX01.0012	Human	8/5/2015
PNUSAS000758	VA___R150800859	JJPX01.0012	Human	8/8/2015
PNUSAS000831	NY___IDR1500058628	JJPX01.0012	Human	8/8/2015
PNUSAS000846	MD___MDA15093460	JJPX01.0012	Human	7/27/2015
PNUSAS000850	MD___MDA15093943	JJPX01.0012	Human	7/25/2015
PNUSAS000865	NY___IDR1500059203	JJPX01.0012	Human	8/17/2015
PNUSAS000888	VA___R150802307	JJPX01.0012	Human	8/25/2015

* 2015K-0753 NJ___151107   Not in Database   Human

hqSNP tree had phages masked, a reference of CFSAN000847, and was generated with Lyve-SET version 1.0.0. Reads were cleaned with CG Pipeline, SNPs were called with Varscan, and Lyve-SET was run with the following options: minimum coverage- 20, min alternative fraction- 0.95, and allowed flanking- 5.
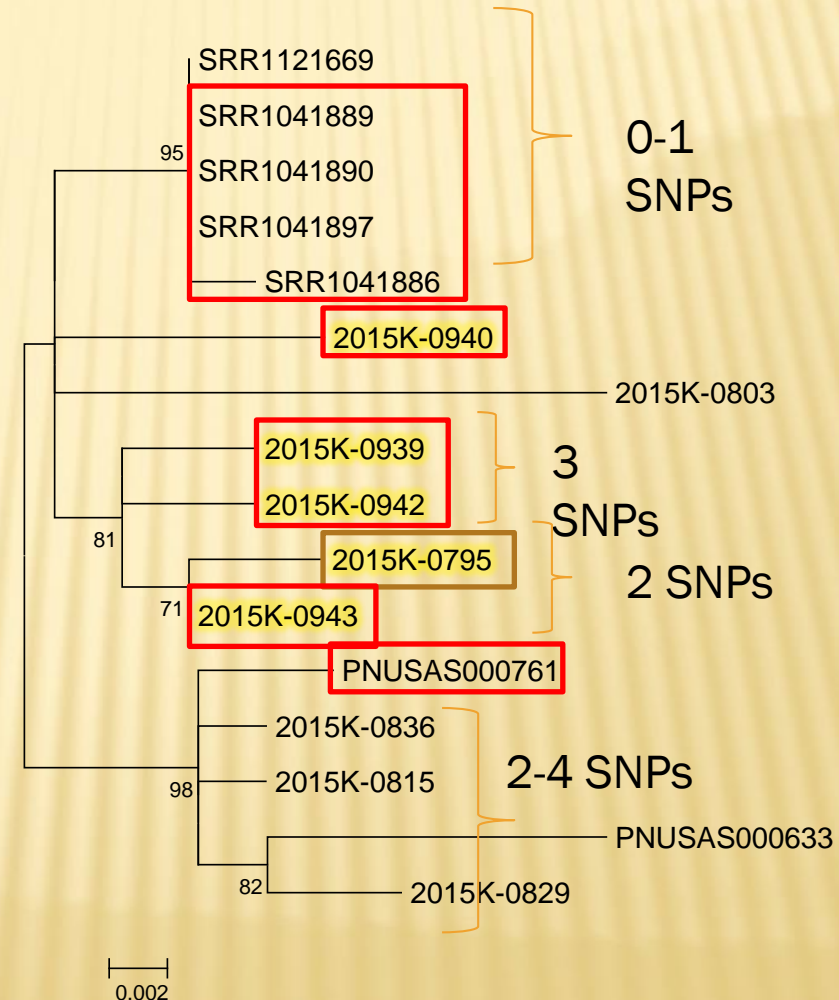
1507MLJJP-1 – DELMARVA STUDY - NEWPORT HQSNP TREE- BOX 6 – JJPX01.0061 CLUSTER (NEW ADDITIONS RED BOXED)

WGS_id	Key	PFGE-XbaI-pattern	SourceSite	IsolatDate
2015K-0795	DE___B629554	JJPX01.0061	Stool	6/29/2015
2015K-0803	MA___15EN4838	JJPX01.0061	Stool	7/2/2015
2015K-0815	PA___M15010457-1	JJPX01.0061	Stool	7/24/2015
2015K-0829	VT___16BAC0039	JJPX01.0061	Stool	7/14/2015
2015K-0836	RI___15P090	JJPX01.0061	Stool	7/29/2015
2015K-0939	DE___B629590	JJPX01.0061	Stool	8/1/2015
2015K-0940	DE___B629592	JJPX01.0061	Stool	8/7/2015
2015K-0942	DE___B629596	JJPX01.0061	Stool	8/10/2015
2015K-0943	DE___B629597	JJPX01.0061	Stool	8/14/2015
PNUSAS000633	MD___MDA15025583	JJPX01.0061	Stool	2/14/2015
PNUSAS000761	NY___IDR1500057193	JJPX01.0061	Stool	8/3/2015
SRR1121669	NA	NA	Cilantro, NY	8/3/2012
SRR1041889	NA	NA	Cilantro, NY	2012
SRR1041890	NA	NA	Cilantro, FL	2012
SRR1041897	NA	NA	Cilantro, FL	2012
SRR1041886	NA	NA	Cilantro, NY	2012

CFSAN000847 used as reference with prophage regions masked; Lyve-SET version 1.0.0; min_coverage=20; min_alt_frac=0.95; allowedFlanking=5; read_cleaner= CG Pipeline; read_mapper=smalt; SNP caller = Varsca

Delmarva Study - Newport hqSNP Tree- Box 2a – JJPX01.0011 cluster (New additions red boxed)

2015K-0755	NJ___151109	NJ	JJPX01.0011		Stool	7/13/2015
2015K-0793	DE___B625216	DE	JJPX01.0011		Stool	6/24/2015
2015K-0796	DE___B629558	DE	JJPX01.0011		Stool	7/10/2015
2015K-0800	DE___B629568	DE	JJPX01.0011		Stool	7/21/2015
2015K-0812	PA___M15010029-1	PA	JJPX01.0011		Stool	7/18/2015
2015K-0813	PA___M15010269-1	PA	JJPX01.1875		Stool	7/20/2015
2015K-0817	PA___M15010365-1	PA	JJPX01.0011		Stool	7/20/2015
2015K-0873	PA___M15010819-1	PA	JJPX01.0011		Stool	7/29/2015
2015K-0889	NJ___151281	NJ	JJPX01.0011		Stool	7/21/2015
2015K-0896	NJ___151318	NJ	JJPX01.0011		Stool	8/3/2015
2015K-0897	NJ___151320	NJ	JJPX01.0011		Stool	8/5/2015
2015K-0937	DE___B624392	DE	JJPX01.0011		Stool	8/1/2015
PNUSAS000597	VA___R150700988	NC	JJPX01.0011		Stool	7/9/2015
PNUSAS000623	VA___R150701197	VA	JJPX01.0030		Stool	7/12/2015
PNUSAS000634	NY___IDR1500053534	NY	JJPX01.0011		Stool	7/11/2015
PNUSAS000679	MD___MDA15078550	NC	JJPX01.0011		Stool	6/25/2015
PNUSAS000717	NY___IDR1500054331	NY	JJPX01.0011		Stool	7/21/2015
PNUSAS000730	VA___R150701926	VA	JJPX01.0011		Stool	7/20/2015
PNUSAS000763	NY___IDR1500057368	NY	JJPX01.0011		Stool	8/4/2015
PNUSAS000795	VA___R150800950	NC	JJPX01.0011		Stool	8/7/2015
PNUSAS000811	NY___IDR1500058331	NY	JJPX01.0011		Urine	8/15/2015
PNUSAS000812	NY___IDR1500058467	NY	JJPX01.0011		Stool	8/8/2015
PNUSAS000815	VA___R150801370	VA	JJPX01.0178		Stool	8/15/2015
PNUSAS000866	MN___E2015012234	MN	JJPX01.0011	1509MNJJP-1	Stool	8/28/2015
PNUSAS000867	MN___E2015012135	MN	JJPX01.0011	1509MNJJP-1	Stool	8/25/2015
PNUSAS000872	MN___E2015010923	MN	JJPX01.0011	1509MNJJP-1	Stool	8/4/2015
PNUSAS000875	VA___R150801893	VA	JJPX01.0011		Stool	8/20/2015
SRR949456	NYAG_367856-4-1		JJPX01.0011		Cilantro	7/8/2008
SRR949455	NYAG_367856-4-1		JJPX01.0011		Cilantro	7/8/2008
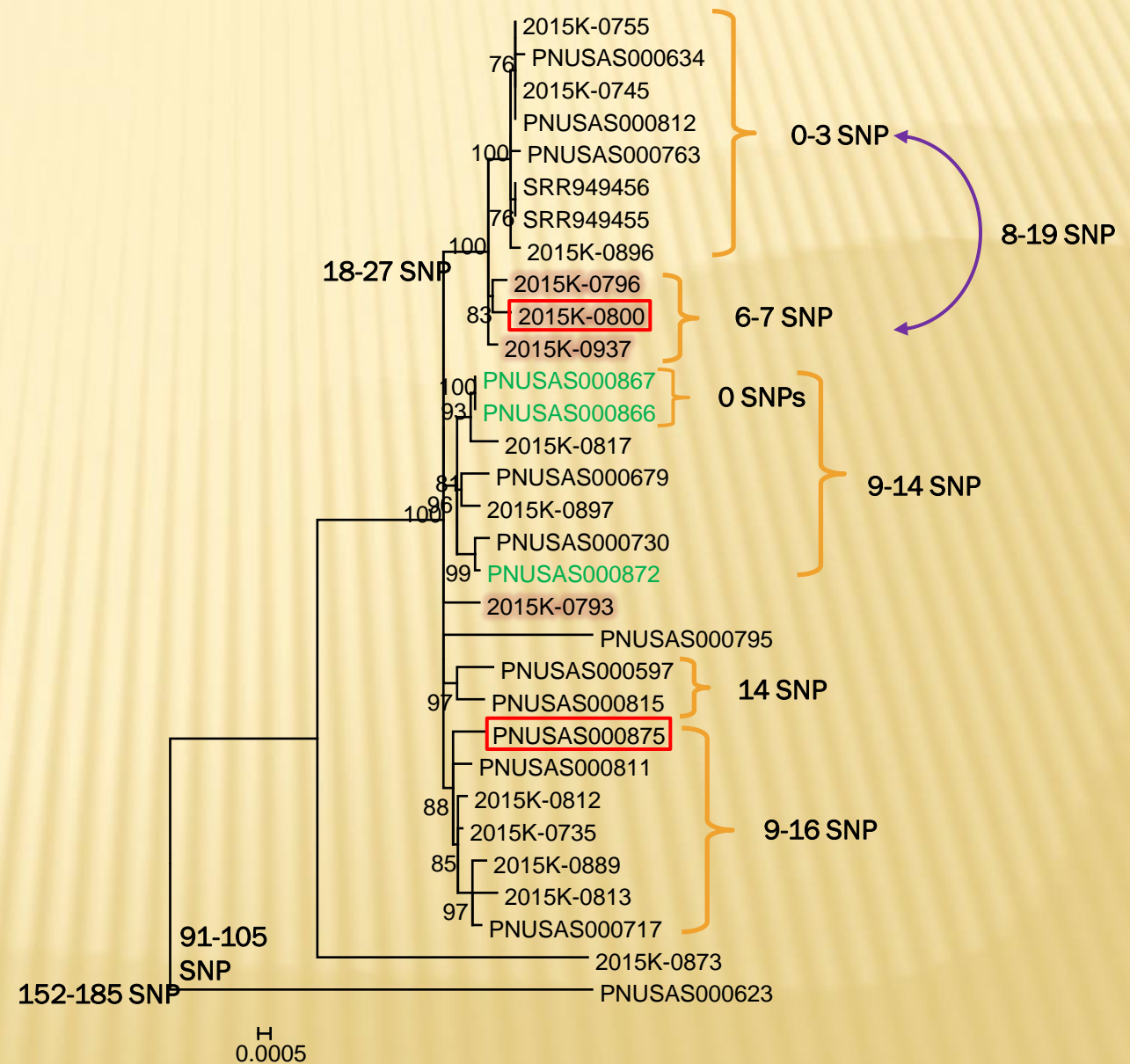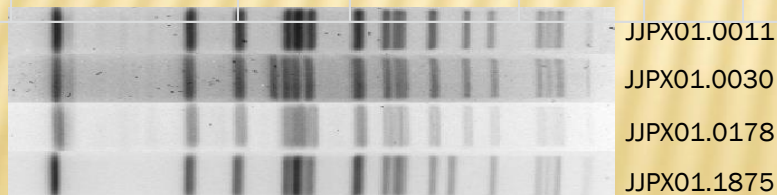
Phages masked, reference of CFSAN000847, and generated with Lyve-SET version 1.0.0. Reads were cleaned with CG Pipeline, SNPs were called with Varscan, and Lyve-SET was run with the following options: minimum coverage- 20, min alternative fraction- 0.95, and allowed flanking- 5.

Overview of the Delmarva Produce Safety Task Force

A Tri-State Collaborative Effort to Address a Regional Issue

Delmarva Produce is Very Important to the U.S.

Table 1. Acreage of Selected Fresh Vegetables from Delaware and counties on the Eastern Shore of Maryland and Virginia.

	Delaware	Maryland	Virginia
Tomatoes	80	250	2,000
Cantaloupe	140	500	10
Watermelons	2,500	2,100	10
Leafy Vegetables*	10	40	40
Cabbage	500	10	10
Cucumbers	10	30	10

*- Includes kale, spinach, and garden lettuces, all grown for very local markets. Head lettuce is not produced on Delmarva.

10/22/2015

Outbreaks Linked to Delmarva Produce

- **Since 2002, Delmarva tomatoes and cucumbers have been implicated in six outbreaks associated with *Salmonella* Newport.**
  - Tomatoes (Virginia): 2002 (333 reported illnesses), 2005 (72 reported illnesses), 2006 (115 reported), 2007 (65 reported), 2010 suspected (51 reported). *(Source: FDA)*
  - Cucumbers (Maryland): 2014 (275 with estimated 51 of these consuming MD cucumbers). *(Source: FDA)*

10/22/2015

Recognizing the importance of addressing the issue, Delaware, Maryland and Virginia officials began working in close partnership with each other and the FDA to better understand the cause of contamination and to ensure adequate food safety measures are in place.

10/22/2015

The Delmarva Produce Safety Task Force

- **Led by the three states, the Delmarva Produce Safety Task Force was formed in April 2015 as a regional, proactive effort to ensure continued close collaboration between the agencies.**
  - Four work groups were formed– Communications, Research, Good Agricultural Practices and Epidemiology/Surveillance.
  - The Task Force is reviewing the science and current research to incorporate any new information into Good Handling Practices and Good Agricultural Practices training sessions to enhance food safety and reduce the risks associated with foodborne pathogens.
  - Delaware, Maryland and Virginia began to work directly with the producers in their own states during this growing season to quickly make producers aware of the issue and provide risk mitigation strategies.

10/22/2015

Collaboration and Coordination

- The task force represents a unique partnership led by the states of Delaware, Maryland and Virginia in partnership with the FDA and other stakeholders
  - The collaborative approach among the appropriate agencies within each state, specifically their agricultural and public health agencies and Extensions, includes coordination of all activities and programs in each state.
- There is a tremendous amount of scientific methodology, process and procedure associated with the execution of each state's plan. This plan represents an adaptive management strategy, with emphasis and strategies changing as the situation warrants.

10/22/2015

Complexities

- *Salmonella* Newport is naturally occurring in the environment and wildlife – including sea gulls and other water fowl, reptiles, surface waters and sediments.
- It is rarely one factor that contributes to an increase in *Salmonella* outbreaks, but usually a combination of contributors.

- The science surrounding the spread of *Salmonella* Newport on the Delmarva is incomplete.
- Research is ongoing to further identify effective and appropriate safety practices based on the latest science.

http://phil.cdc.gov/phil/details.asp?pid=10896

10/22/2015
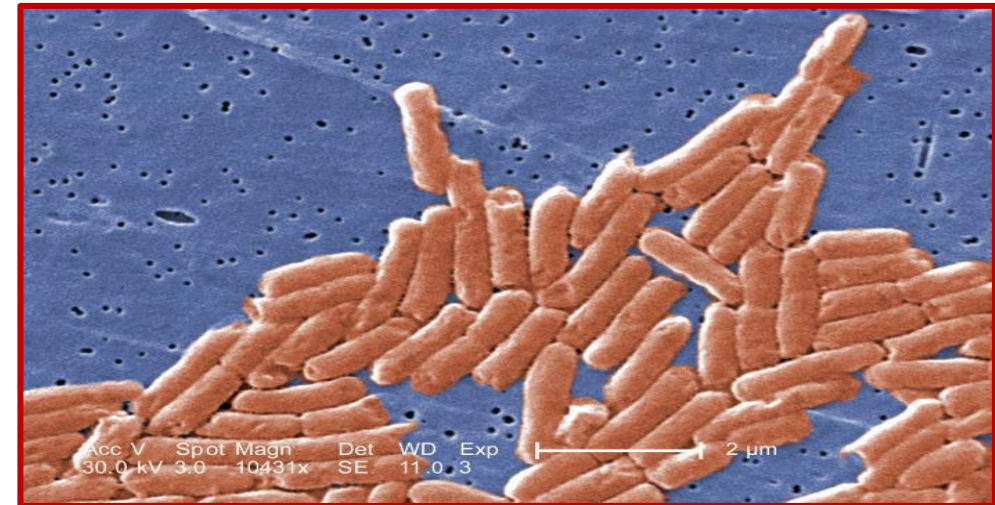
Next Steps

- **An Action Plan has been developed by the states and will continue to evolve as new information is learned.**
  - Each state has individual activities as well as collaborations.
  - FDA will continue its research in this area.
- **The Task Force has been in regular communication throughout this growing season and, to date, no outbreaks have been linked to Delmarva produce in 2015.**
- **The collaboration is committed to closing knowledge gaps and mitigating the risks of *Salmonella* contamination of produce on the Delmarva.**

10/22/2015

SAFE DELMARVA PRODUCE

IS THE GOAL!

10/22/2015

ENVIRONMENTAL LABORATORY SAMPLES RECEIVED FROM THE DELAWARE DEPARTMENT OF AGRICULTURE

1. Environmental sponges from packing lines (before and after disinfection)
2. Pond/Surface Waters

BASIC COLLECTION/PROCESSING OF THE ENVIRONMENTAL SAMPLES

- Packing Lines: Using sterile technique, swipe a 1ft x 1ft area with the sponge containing DE Neutralizing Broth. Placed in a sterile whirlpac bag. Refrigerate and submit to DPHL within 24 hrs of collection.
  - Remove sponge and place in primary enrichment (225mL of lactose broth) Incubate overnight at 35°C.
  - Remove an aliquot of primary enrichment and add to two secondary enrichment medias (RV and TT w/iodine). Incubate overnight at 42°C.
  - Streak secondary enrichments onto selective and differential medias (HE and XLD) to look for Salmonella species.

BASIC COLLECTION/PROCESSING OF THE ENVIRONMENTAL SAMPLES

✖ Pond/Surface Waters: Collect 1L of water without disturbing sediment. Refrigerate and transport to DNREC within six hours of collection.

　✚ Filter all of water, changing filters as necessary. Place filters into 200mL of Buffered Peptone Water. Incubate overnight at 35°C.

　✚ Remove an aliquot and add to two secondary enrichment medias (RV and TT with iodine). Incubate overnight at 42°C.

　✚ Streak secondary enrichments onto selective and differential medias (HE and XLD) to look for Salmonella species

IDENTIFICATION OF SALMONELLA SPECIES

1. Pick at least three suspicious colonies from each agar plate and re-streak to sheep blood and MacConkey agars. If colony is a non-lactose fermenter (NLF) and not swarming, performed identification on the Vitek 2.

2. Group all Salmonellas using serological methods (somatic O-antigen).

3. Salmonella Newport, group C_2, NOT present in any environmental samples.